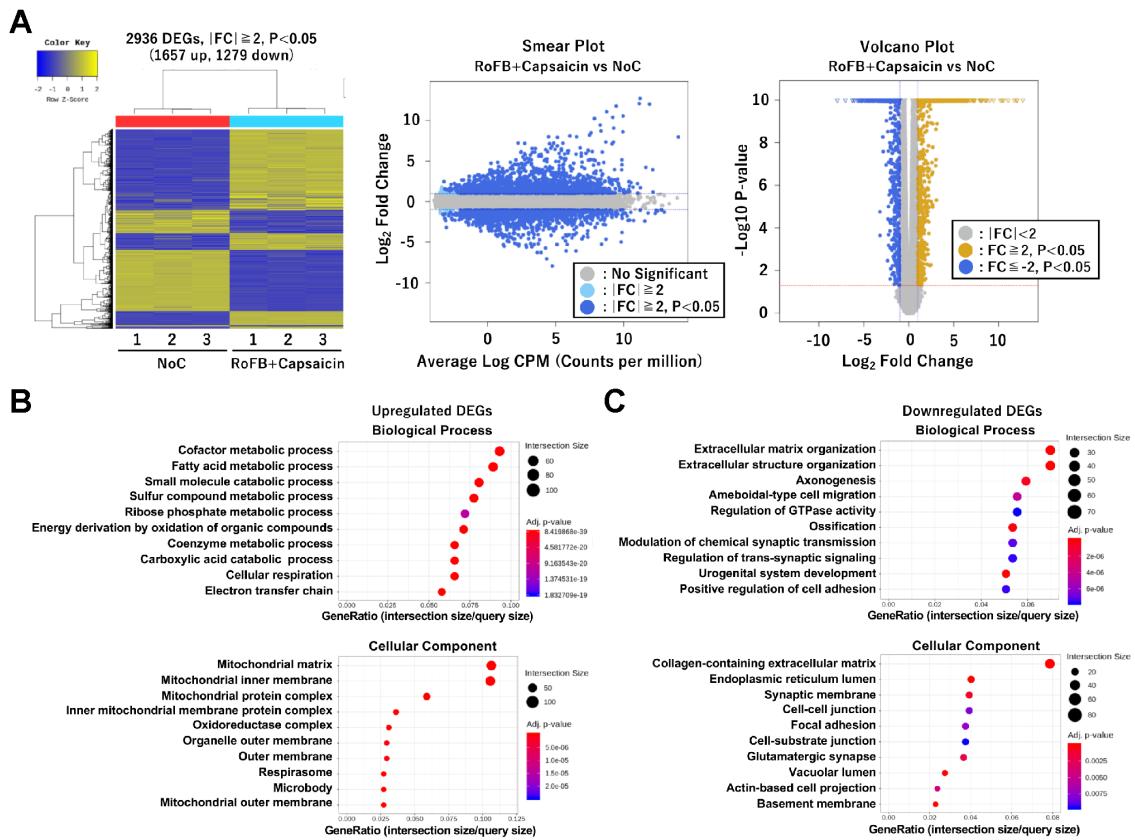


Capsaicin directly promotes adipocyte browning in the chemical compound-induced brown adipocytes converted from human dermal fibroblasts

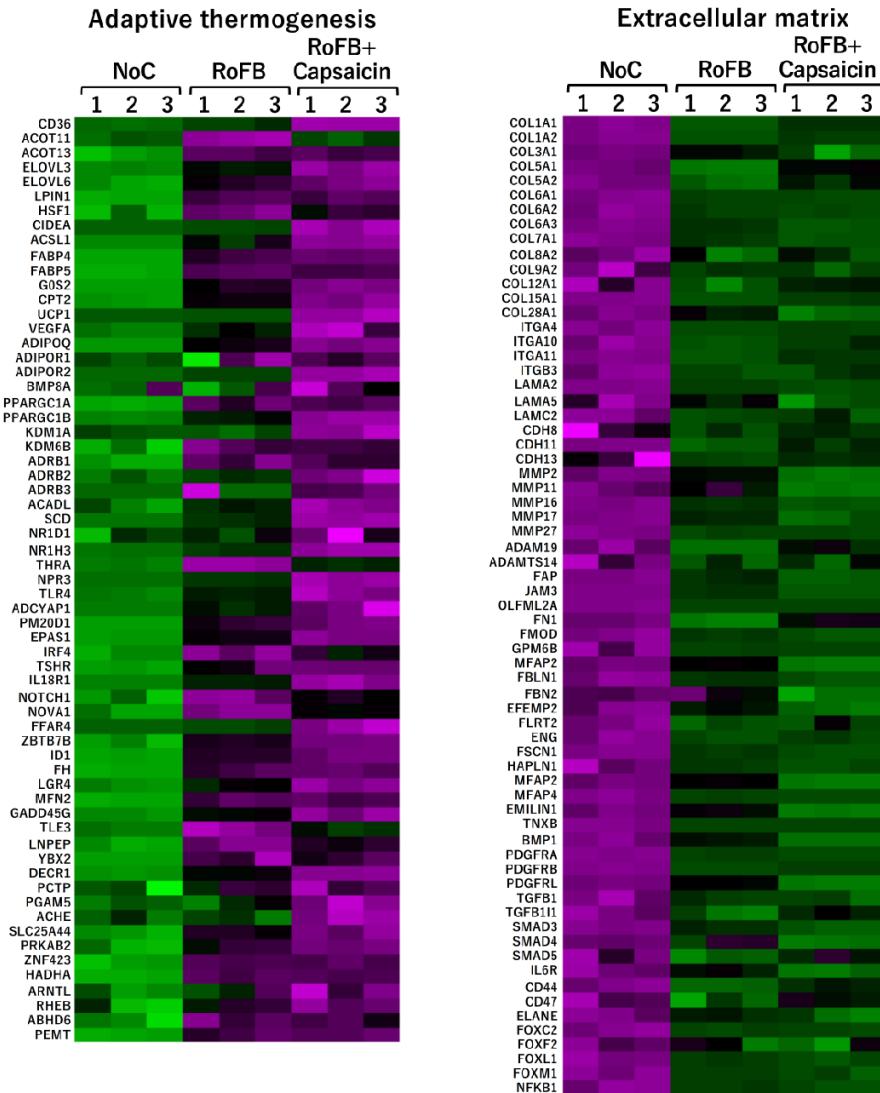
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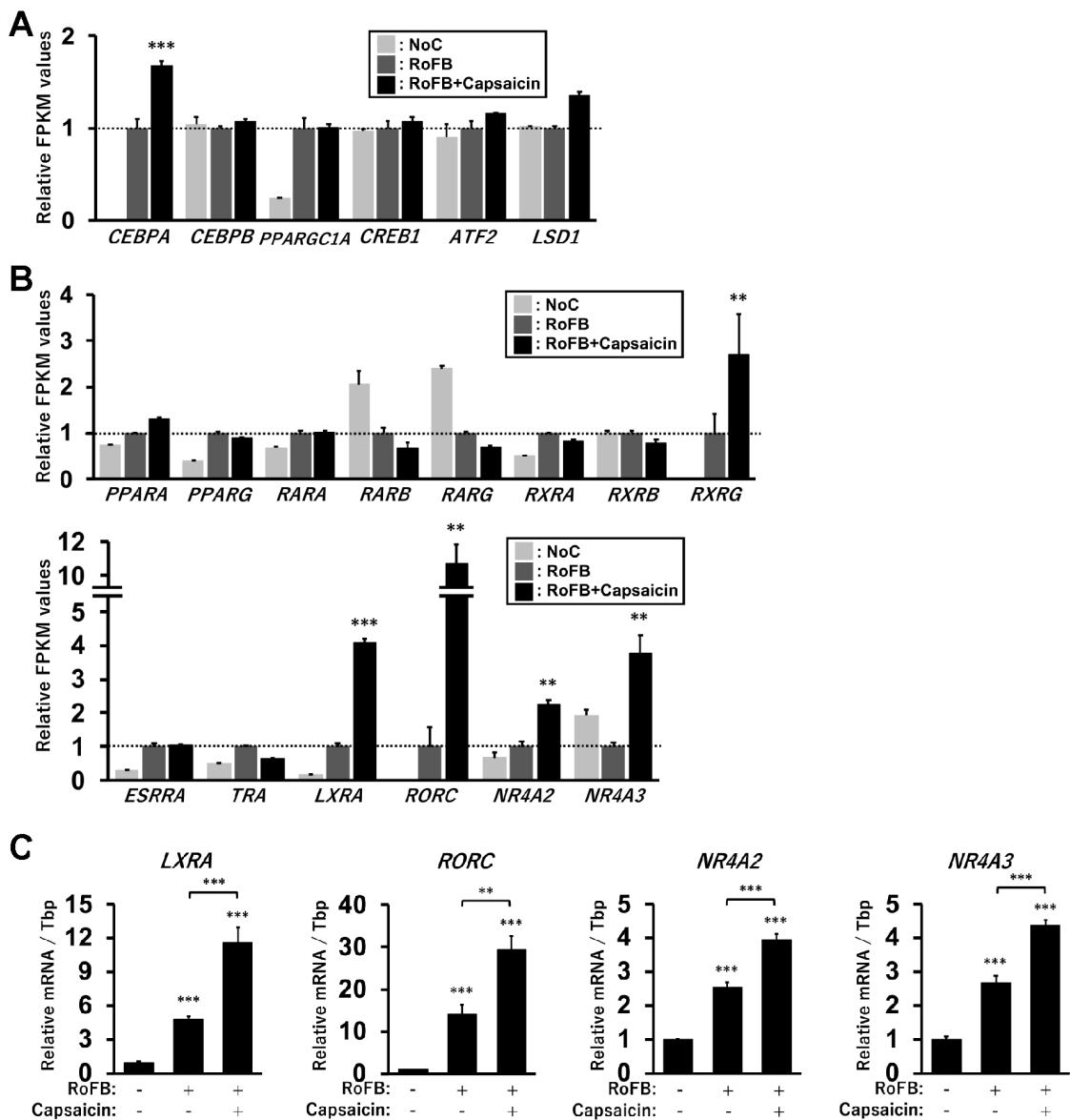
Supplementary Information



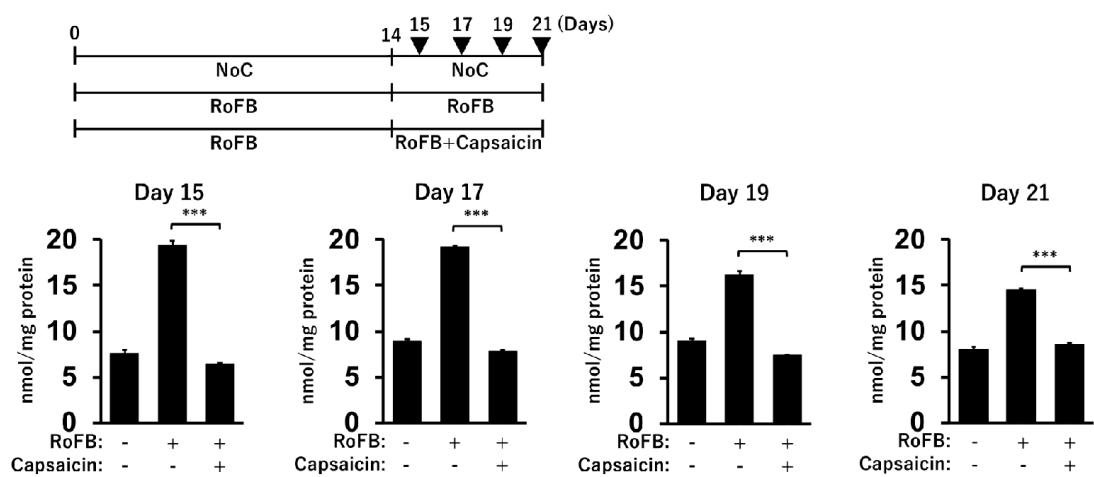
Supplementary Figure S1. Comparison of genome-wide transcriptional analysis between Capsaicin-treated ciBAs (RoFB+Capsaicin) and the control fibroblasts (NoC) cultured in the SFBAM only in parallel. **(A)** Heat map and hierarchical clustering analysis represent 2936 differentially expressed genes (DEGs) ($|fold\ change\ (FC)| \geq 2, P < 0.05$) between NoC and RoFB+Capsaicin. Smear and Volcano plots indicate logarithmic fold change, P-value, and CPM (counts per million) between them. **(B,C)** Gene ontology (GO) enrichment analysis was performed in the 1657 up- and 1279 down-regulated DEGs. Top 10 GO terms are represented in the category of Biological Process and Cellular Component.



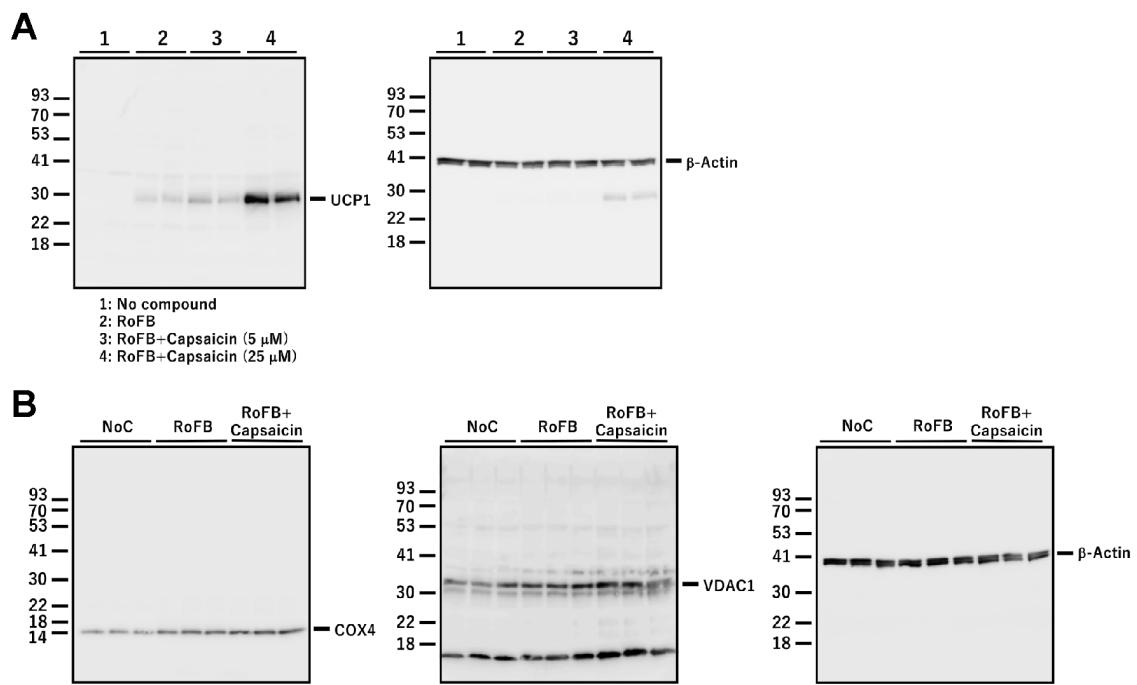
Supplementary Figure S2. Heat maps represent transcriptional profiles of functional genes related to adaptive thermogenesis and extracellular matrix. The color scale shows z-scored fragments per kilobase of transcript per million mapped sequence reads (FPKM) representing mRNA levels of each gene in green (lower expression) and magenta (higher expression).



Supplementary Figure S3. The expression of transcription factors and nuclear receptors in Capsaicin-treated ciBAs. **(A,B)** Relative FPKM values obtained from the RNA-Seq results were shown in transcription factors (**A**) and nuclear receptors (**B**) which are involved in the regulation of *UCP1* and metabolic gene expression. **(C)** The expression of *LXRA*, *RORC*, *NR4A2*, and *NR4A3* was quantified by qRT-PCR analysis in the control, ciBAs, and Capsaicin-treated ciBAs. Data represent mean \pm SD (n=3). Student's t-test: **P<0.01, ***P<0.001.



Supplementary Figure S4. Glycerol secretion after the treatment with Capsaicin according to an experimental scheme. The secretion of glycerol into the culture medium was measured at Day 15, Day 17, Day 19, and Day 21 in the control cells (NoC), ciBAs (RoFB), and ciBAs treated with Capsaicin (RoFB+Capsaicin) from Day 14. Data represent mean \pm SD. Student's t-test: ***P<0.001.



Supplementary Figure S5. Images of full-length western blot. **(A,B)** The raw results of western blot analysis are shown for Figure 1D **(A)** and Figure 2C **(B)**.

Supplementary Table S1. List of representative metabolic genes upregulated by Capsaicin in ciBAs.

Gene name	Description	Fold Change	Adjusted p-value
<i>UCP1</i>	Uncoupling protein 1	43.71	0.00E+00
<i>PLA2G2A</i>	Phospholipase A2 group IIA	30.21	0.00E+00
<i>ABCG1</i>	ATP binding cassette subfamily G member 1	21.95	1.60E-207
<i>LIPG</i>	Lipase G, endothelial type	20.84	8.50E-100
<i>SLC2A5</i>	Solute carrier family 2 member 5	16.28	5.39E-136
<i>MOGAT1</i>	Monoacylglycerol O-acyltransferase 1	14.74	1.47E-62
<i>CIDEA</i>	Cell death inducing DFFA like effector a	10.81	2.78E-189
<i>FFAR4</i>	Free fatty acid receptor 4	10.18	4.19E-44
<i>ALDH5A1</i>	Aldehyde dehydrogenase 5 family member A1	9.31	2.97E-87
<i>ACSL5</i>	Acyl-CoA synthetase long chain family member 5	8.64	7.39E-118
<i>ABCC2</i>	ATP binding cassette subfamily C member 2	7.86	8.44E-128
<i>LIPM</i>	Lipase family member M	7.48	2.98E-17
<i>MGAT3</i>	Beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase	6.90	2.04E-79
<i>ME1</i>	Malic enzyme 1	6.83	5.36E-146
<i>PFKFB1</i>	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	6.55	2.31E-126
<i>GLYCTK</i>	Glycerate kinase	6.29	2.45E-20
<i>CKMT1A</i>	Creatine kinase, mitochondrial 1A	6.21	3.47E-69
<i>ATPIA4</i>	ATPase Na+/K+ transporting subunit alpha 4	5.95	1.24E-41
<i>GPAM</i>	Glycerol-3-phosphate acyltransferase, mitochondrial	5.20	2.11E-62
<i>PCK2</i>	Phosphoenolpyruvate carboxykinase 2, mitochondrial	5.15	4.56E-159
<i>CD36</i>	CD36 molecule	5.06	5.93E-95
<i>LINCA DL</i>	LineRNA adipogenesis and lipogenesis associated	5.02	2.75E-57
<i>CKMT1B</i>	Creatine kinase, mitochondrial 1B	4.87	8.24E-96
<i>ACSS2</i>	Acyl-CoA synthetase short chain family member 2	4.36	3.70E-109
<i>LPL</i>	Lipoprotein lipase	4.26	1.05E-118
<i>PNPLA3</i>	Patatin like phospholipase domain containing 3	4.19	2.28E-93
<i>ACSL4</i>	Acyl-CoA synthetase long chain family member 4	3.77	1.21E-50
<i>SCD</i>	Stearoyl-CoA desaturase	3.71	4.18E-93
<i>FAR2</i>	Fatty acyl-CoA reductase 2	3.69	5.17E-114
<i>SLC2A4</i>	Solute carrier family 2 member 4	3.65	6.23E-76
<i>HMGCS1</i>	3-Hydroxy-3-methylglutaryl-CoA synthase 1	3.50	1.15E-34
<i>CYP4F12</i>	Cytochrome P450 family 4 subfamily F member 12	3.48	1.84E-09
<i>LDHD</i>	Lactate dehydrogenase D	3.46	1.15E-80
<i>ACLY</i>	ATP citrate lyase	3.38	3.14E-87
<i>NMNAT2</i>	Nicotinamide nucleotide adenylyltransferase 2	3.31	1.39E-17
<i>KCNK3</i>	Potassium two pore domain channel subfamily K member 3	3.26	9.34E-95

<i>BBOXI</i>	Gamma-butyrobetaine hydroxylase 1	3.17	9.52E-05
<i>INSIG1</i>	Insulin induced gene 1	3.14	1.13E-63
<i>GSTA1</i>	Glutathione S-transferase alpha 1	3.04	7.16E-26
<i>ECHDC1</i>	Ethylmalonyl-CoA decarboxylase 1	3.02	9.83E-68
<i>SPHK1</i>	Sphingosine kinase 1	2.96	5.45E-53
<i>GK</i>	Glycerol kinase	2.83	2.86E-44
<i>SREBF1</i>	Sterol regulatory element binding transcription factor 1	2.82	1.96E-65
<i>ADCYAP1</i>	Adenylate cyclase activating polypeptide 1	2.81	1.65E-08
<i>LPIN3</i>	Lipin 3	2.79	1.92E-53
<i>ADIPOR2</i>	Adiponectin receptor 2	2.75	3.69E-70
<i>CKMT2</i>	Creatine kinase, mitochondrial 2	2.72	4.96E-48
<i>MGLL</i>	Monoglyceride lipase	2.71	6.71E-64
<i>ELOVL5</i>	ELOVL fatty acid elongase 5	2.58	2.39E-60
<i>ADRB2</i>	Adrenoceptor beta 2	2.58	1.98E-12
<i>NAMPT</i>	Nicotinamide phosphoribosyltransferase	2.55	3.96E-46
<i>FADS2</i>	Fatty acid desaturase 2	2.54	2.25E-53

Supplementary Table S2. List of representative genes downregulated by Capsaicin in ciBAs.

Gene name	Description	Fold Change	Adjusted p-value
<i>ACAN</i>	Aggrecan	-53.52	1.46E-304
<i>ADRA1A</i>	Adrenoceptor alpha 1A	-38.80	2.72E-180
<i>CDH23</i>	Cadherin related 23	-28.29	1.09E-92
<i>PRIMA1</i>	Proline rich membrane anchor 1	-14.72	2.05E-33
<i>ROBO2</i>	Roundabout guidance receptor 2	-14.55	1.56E-30
<i>CNTN1</i>	Contactin 1	-13.50	5.01E-30
<i>GALNT17</i>	Polypeptide N-acetylgalactosaminyltransferase 17	-11.44	1.05E-47
<i>GPRC5B</i>	G protein-coupled receptor class C group 5 member B	-11.03	2.32E-174
<i>PCDH20</i>	Protocadherin 20	-11.02	7.25E-92
<i>TRPV3</i>	Transient receptor potential cation channel subfamily V member 3	-10.66	6.43E-11
<i>NCAM1</i>	Neural cell adhesion molecule 1	-8.77	2.03E-86
<i>TTR</i>	Transthyretin	-7.91	1.51E-68
<i>F2RL2</i>	Coagulation factor II thrombin receptor like 2	-7.20	2.46E-17
<i>CDH20</i>	Cadherin 20	-7.18	1.04E-40
<i>MSR1</i>	Macrophage scavenger receptor 1	-7.06	9.72E-10
<i>FGFR2</i>	Fibroblast growth factor receptor 2	-7.03	5.47E-102
<i>PCDHGA11</i>	Protocadherin gamma subfamily A, 11	-6.82	2.07E-17
<i>TRPV2</i>	Transient receptor potential cation channel subfamily V member 2	-6.69	2.25E-23
<i>TRPM1</i>	Transient receptor potential cation channel subfamily M member 1	-6.61	5.97E-05
<i>AEBP1</i>	AE binding protein 1	-6.59	1.24E-166
<i>P2RY14</i>	Purinergic receptor P2Y14	-6.54	5.64E-14
<i>AQPI</i>	Aquaporin 1 (Colton blood group)	-6.47	1.44E-136
<i>CDH22</i>	Cadherin 22	-5.76	9.49E-09
<i>ST6GAL1</i>	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	-5.65	2.21E-21
<i>BGN</i>	Biglycan	-5.32	2.95E-54
<i>CILP2</i>	Cartilage intermediate layer protein 2	-5.29	2.56E-58
<i>SGCG</i>	Sarcoglycan gamma	-5.21	9.26E-93
<i>MFRP</i>	Membrane frizzled-related protein	-4.79	3.84E-02
<i>MMP11</i>	Matrix metallopeptidase 11	-4.78	3.63E-12
<i>GPC3</i>	Glypican 3	-4.77	4.59E-82
<i>SGCA</i>	Sarcoglycan alpha	-4.70	2.35E-23
<i>POSTN</i>	Periostin	-4.70	9.17E-99
<i>MMP7</i>	Matrix metallopeptidase 7	-4.69	3.62E-44
<i>ADRA2A</i>	Adrenoceptor alpha 2A	-4.48	3.46E-72
<i>MATN2</i>	Matrilin 2	-4.31	5.54E-68
<i>COMP</i>	Cartilage oligomeric matrix protein	-4.29	3.28E-51

<i>ST8SIA4</i>	ST8 alpha-N-acetyl-neuraminate alpha-2,8-sialyltransferase 4	-4.11	2.43E-06
<i>ADAM28</i>	ADAM metallopeptidase domain 28	-4.10	1.98E-06
<i>CSGALNACT1</i>	Chondroitin sulfate N-acetylgalactosaminyltransferase 1	-4.10	1.57E-77
<i>NTN1</i>	Netrin 1	-4.03	1.51E-131
<i>LEPR</i>	Leptin receptor	-3.36	4.22E-68

Supplementary Table S3. Sequences of primers used for qRT-PCR.

Gene	Sense primer	Antisense primer
<i>TBP</i>	ACTACGGGGTTATCACCTGTGAG	GTGCAGGAGTAGGCCACATTAC
<i>UCP1</i>	TCTACGACACGGTCCAGGAG	GAATACTGCCACTCCTCCAGTC
<i>FABP4</i>	GCCAGGAATTGACGAAGTCA	CCCATTCTGCACATGTACCAG
<i>MT-CYB</i>	ACTCCACCTCCTATTCTTGAC	TGTGTAGTAAGGGTCCAAGGTG
<i>MT-ND5</i>	CTTAGGCGCTATCACCACTCTG	CTTGAAGTGGAGAAGGCTACGA
<i>TRPV1</i>	GCTGTCTTCATCATCCTGCTG	TTGCTCTCCTGTGCGATCTTG
<i>TRPV2</i>	TTCCTTTCGGCTTCGCTGTAG	GCACTGACTCTGTGGCATTG
<i>TRPV3</i>	CTCCTCAACATGCTATTGCTC	TCTCAAACCTCAAGATGGCCTG
<i>TRPV4</i>	GCTCGTCTACTTGCTCTTCATG	CAGTTGGTCTGGTCCCTCATTG
<i>CIDEA</i>	AAGGCCACCATGTATGAGATGTAC	ACAGGAACCGCAGCAGACTC
<i>LIPG</i>	CACCCTTATGGCACTAACATGCAG	GAAGGTGTTGGTGGCATTCTG
<i>CKMT1</i>	AGCAGGAATGGCTCGAGAC	ATCCTCCTCATTCACCCAGATC
<i>CD36</i>	CAACCTATTGGTCAAGCCATCAG	TGTTTGCCTTCTCATCACCAATG
<i>SCD</i>	ACTTGTCTGACCTAGAACGCTGAG	GATGAAGCACATCATCAGCAAG
<i>KCNK3</i>	CCATCACCGTCATCACAC	CAGCGCGTAGAACATGCAG
<i>SREBF1</i>	CCTGGTCTACCATAAGCTGCAC	CTGCACACTCTGCCAGGTT
<i>FASN</i>	CCTTCGAGGTGTCAGAGAAC	TTTCCGGGTGGTCAAGAG
<i>PLIN1</i>	AGCATTGAGAAAGGTGGTGGAG	ACTTCTGGGCTTGCTGGTG
<i>LIPE</i>	CTCAGTGTGCTCTCCAAGTG	CTTTCTGGTCTGAGTTGGAGTG
<i>ADIPOQ</i>	CTGGTGAGAAGGGTGAGAAAG	GTTCACCGATGTCTCCCTAG
<i>AEBPI</i>	AACGCCACCATCTCTGTGAG	CAAGATTGCCAGTAATCACAC
<i>CEBPA</i>	AACATCGCGGTGCGCAAGA	AGCTCCAGCACCTCTGCT
<i>PPARGC1A</i>	AGGTCAAGATCAAGGTCTCCAG	GGTGTCTGTAGTGGCTTGACTC
<i>PPARG</i>	TGGAATTAGATGACAGCGACTTG	CTTCAATGGGCTTCACATTAG
<i>RXRG</i>	GGCCTACACCAAGCAGAAGTATC	CAAGCCAATGGAACCGCAGAG
<i>GK</i>	GATTGTCTGCCGTACGATG	CCAACCCATTGACTTCATCACAG
<i>GPAM</i>	GATCGCATTATCGAAGGTCACTAC	TTGCTACACTCCACAGGCTCTC
<i>AGPAT2</i>	CCCGTGGTGTACTCTCCTTC	TTCCAGCACCTGCACTGTGA
<i>LPIN3</i>	CATCCTCTGTCTCCCAGCAG	GGCGACCTTGAACACCTCTG
<i>DGPAT2</i>	TACTCCAAGCCCACACAC	ATGGTGTGGTACAGGTCATG
<i>AQP7</i>	CTGCCACCATCTACAGTCTCTC	TCATGTGATCAGGAAGGTAGGTG
<i>GPD1</i>	CTGACCTGATCACTACCTGCTATG	GCAACTCTTCTCCAGCTGCTC
<i>LXRA</i>	CAGATTGCCCTGCTGAAGAC	AGGGTTGTACCTCCGAGATG
<i>RORC</i>	GAGGATGAGATTGCCCTCTACAC	GGCCAGCTCCAGATTGTACTG
<i>NR4A2</i>	AACTTGAGAATATGAACATCGAC	GTTCTCCACTCTTGGGTT
<i>NR4A3</i>	TTGGAGCTTTGTCCCTCAGAC	ACTGAAGTCGATGCAGGACAAG